May 1997

			REVISED FINAL

Aquatic Effects Technology Evaluation Program

Project #3.1.2.
Technical Evaluation:
Water Quality and Biological Effects

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101-02560/

AQUATIC EFFECTS TECHNOLOGY EVALUATION PROGRAM

PROJECT #3.1.2. TECHNICAL EVALUATION: Water Quality and Biological Effects

REVISED FINAL REPORT

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3/729-02

May 1997

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ACKNOWLEDGEMENTS

EVS Environment Consultants thank the AETE Technical Committee for the opportunity to write this Technical Evaluation. Peter Kiffney, Alena Fikart and Erika Szensay wrote the draft evaluation. The revised report was prepared by Peter Kiffney, Alena Fikart, Margot Daykin and Howard Bailey. Peter Chapman reviewed the document which was word processed by Sue Bate and produced by Rhea Sichani. We are grateful to three referees nominated by the AETE Technical Committee, whose comments appreciably improved an earlier draft: Gwendy Hall, Marcia Blanchette and Larry Pommen. This project was co-ordinated for the AETE Technical Committee by Madona Skaff.

EXECUTIVE SUMMARY

This evaluation reviewed the relationship between water quality and biological effects in freshwater ecosystems.

The current definition of dissolved metals as the metals component of a water sample that passes through a 0.45 μm filter is inadequate. Specifically, recent research has shown that substantial amounts of metal bound to colloids can pass through this pore size, and thereby can be analyzed as part of the dissolved fraction. The toxicity of these colloid-bound metals is not known, and research is needed to determine their bioavailability and toxicity. We recommend that a technical workshop be convened that addresses what constitutes the dissolved metal fraction, the potential implications of this definition on toxicological effects in the receiving environment, and evaluates various field techniques (e.g., filter type and apparatus) that potentially will minimize contamination of field collected water samples at mine sites. This workshop could also set the basis for future research priorities.

A myriad of abiotic and biotic factors influence the bioavailability and toxicity of metals to aquatic organisms. Furthermore, the relationship between factors that affect toxicity are not always linear. For example, low pH can either increase or decrease toxicity of metals in freshwater ecosystems. Although the dissolved metal fraction, specifically the free metal ion, best predicts toxicity in well-defined synthetic media, this relationship appears to break down in natural waters. This is especially true when one evaluates the influence of organic matter on metal toxicity. We recommend that research be conducted that addresses the relationship of dissolved and total metals and toxicity to aquatic organisms using natural receiving water.

There are a number of modeling approaches that may be of use to monitoring metals in Canadian receiving environments. For example, the U.S. EPA has developed metal translators and conversion factors that convert dissolved metal levels to total recoverable metal levels. In addition, there are a number of chemical equilibrium models that have the potential to be valuable tools in metal monitoring. However, for these models to be useful in routine biological monitoring they need to develop some predictive capacity. For example, can output from one of these models that is based on the chemical characteristics of a mine effluent and the receiving environment be used to predict the toxicity of that effluent? This approach would require using these models in conjunction with toxicity testing to develop a predictive approach to biological monitoring.

In Canada, a frequently cited goal is to analyze trace metals in receiving waters at detection limits equal to or lower than 1/10 of the corresponding CCME (or provincial)

water quality guidelines. We recommend that a risk based approach be used to establish detection limits on a site-specific basis. Such an approach would incorporate the chemistry of the effluent and receiving environment (the use of equilibrium models could be used here) to determine the relative risk of metals of concern in relation to their biological effects and required detection limits. For analytes of concern, detection limits should be based on analytical requirements needed to ensure that reported non-detects are significantly different than appropriate effect concentrations (i.e., generic criteria if applicable or site-specific criteria).

Current technologies analyze total metal levels, whereas the free metal ion may be the most important species in terms of toxicity. There are analytical techniques that characterize free metal ion concentrations in water, such as anodic stripping voltammetry and ion selective electrodes. While these approaches can measure the free metal species, they have a number of problems that hinder their general use for monitoring metals in Canadian receiving environments. For example, anodic stripping voltammetry is time consuming, has a fairly high level of interference and requires a high level of operator expertise. Although ion selective electrodes are susceptible to interference problems in more saline waters, this technique offers the most promise as an analytical tool for monitoring metals in the environment. Further development of alternative analytical techniques is required before they are useful for routine monitoring programs.

1.0
INTRODUCTION

The Aquatic Effects Technology Evaluation (AETE) program was established to review appropriate technologies for assessing the impacts of mine effluents on the aquatic environment. AETE is a cooperative program between the Canadian mining industry, several federal government departments and a number of provincial governments; it is coordinated by the Canadian Center for Mineral and Energy Technology (CANMET). The program is designed to be a direct benefit to industry and government by identifying cost effective technologies to meet environmental monitoring requirements. The program includes three main areas: acute and sublethal toxicity testing, biological monitoring in receiving waters, and water and sediment monitoring. This technical evaluation (#3.1.2) reviews water quality monitoring technology as it relates to measuring biological effects in the receiving environment.

The specific objectives of this evaluation were to:

- Based on available data, address the use of dissolved and total metals analyses in evaluating and/or predicting the aquatic environmental effects of mine effluents. We also addressed the definition of "dissolved" metals. In other words: is the current operational definition of dissolved (i.e., water that passes through a 0.45 μm pore size filter) adequate?; and is there a correlation between filter pore size and bioavailability of metals to aquatic organisms?

- Address the need for ultra-low detection limits in routine monitoring to detect changes in water quality related to measurable biological effects in the receiving environment.

- Identify promising cost-effective alternatives to total and/or dissolved metals analyses which would provide a better indication of metal bioavailability in the aquatic environment downstream from Canadian mining operations.

We evaluated these objectives by critically reviewing the literature. When possible, we present recommendations on methods and technologies that seem the most likely to improve upon current methods used to monitor the effects of metals on freshwater environments. This review deals solely with metals in water and does not discuss the ecotoxicology of metals in sediment.

2.0
TECHNICAL EVALUATION

1.1 Definition of Dissolved Metals

In this review, we define total metals as the metals component of a water sample (including suspended metals) that is acidified to a pH <2 before analysis. Dissolved metals are defined as the metals component of a water sample that is passed through a filter (typically 0.45 μm) and acidified to a pH <2 prior to analysis. Most studies that partition dissolved and particulate metal fractions have relied on filtration through membranes with a 0.45 μm pore size. This approach is adequate for solutes that tend to partition to the aqueous phase in systems without iron (Fe) colloids (Kimball et al., 1995). It has been known for some time that colloidal material passes through 0.45 μm pore size (Jones et al., 1974). For example, aluminum (Al), iron (Fe), and manganese (Mn), require filtration with membranes that have a pore-size smaller than 0.45 μm to adequately define the dissolved fraction (Jones et al., 1974). In Fe-rich systems, even 0.1 μm membranes may allow Fe-colloids to pass through and be analyzed as part of the dissolved phase (Kimball et al., 1992). For example, a water quality study at the Arkansas River, Colorado, USA, a system dominated by Fe-colloids, found that water passed through a 0.45 μm filter contained substantial amounts of colloidal bound arsenic (As), cadmium (Cd), copper (Cu), manganese (Mn), lead (Pb), and zinc (Zn). In addition, these metal bound colloidals occurred in the filtrate defined as Adissolved@ at concentrations that could be potentially toxic to aquatic organisms (Kimball et al., 1995). These data show that substantial amounts of toxic metals bound to the colloid phase can pass through pore size membranes and thereby be analyzed as part of the dissolved fraction. The toxicity of these colloid bound metals is not known and research is needed to determine their bioavailability and toxicity.

Recent studies have shown that a number of factors associated with filtration of water samples other than pore size can produce significant variation in concentrations of metals in the dissolved fraction (Horowitz et al., 1996): the diameter of the filter, the manufacturer, volume of sample processed, and amount of sediment in the sample. For the most part, metals associated with colloidal materials resulted in the variation observed for dissolved metals (Horowitz et al., 1996). Some of the implications of this study include: (1) Regulators should be aware of the potential that different filtration procedures can produce significant variation in dissolved metal levels; (2) Studies that attempt to describe spatial and temporal trends in metal levels are based on data from several sources and the underlying assumption behind this work is that samples processed through any 0.45 μm filter will produce similar results. However, results from the Horowitz et al. (1996) study clearly call this assumption into question; and (3) Filtration artifacts are likely to remain a

problem as long as the environmental/chemical/regulatory community continues to define the dissolved chemical concentration on the basis of physical separation. Horowitz et al. (1996) describe three approaches to minimize filtration artifacts, two of which require major changes to the current operational definition. Artifacts can be minimized by using filters with high surface areas (e.g., capsule filters) and collecting the initial aliquots to quantify artifact-affected constituents. This approach would not entail large-scale changes in current approaches to filtration and is the framework for the new United States Geological Survey=s protocols. Another option would be to pretreat (e.g., filtration, centrifugation) the water to reduce artifact-induced differences in trace element concentrations. While this method would permit the continued use of any 0 .45 μm filter, the pretreatments enhance the likelihood for random contamination due to increased sample handling. More importantly, this approach would significantly lower a number of trace element concentrations in filtrates. The final option is based on the idea that colloidal material represents a contamination that should be avoided from all samples. Depending upon the definition of what constitutes a colloidal material, this would require removing material in the water coarser than at least 0.015-0.005 μm which is 30-90 times finer than at present. This approach would require the use of multiple filters, more expensive equipment, or the use of exhaustive filtration. The most logical approach is to use large surface area membrane filters. Furthermore, a detailed description of all sampling and processing procedures is needed with the publication and/or storage of any chemical data from filtered water (Horowitz et al., 1996).

Taylor and Shiller (1995) also showed that substantial amounts of colloidal material can pass through a 0.45 μm pore size filter resulting in higher dissolved metal concentrations due to insufficient filtration. They recommend the following steps to limit contamination of metal samples: (1) a review of the suitability of all materials that could come into contact with a sample; (2) strict acid cleaning of all apparatus; (3) minimizing sample handling, including possible substitution of grab samples for depth and width integrated samples where appropriate, in well-mixed reaches and (4) exhaustive filtration of samples using a 0.4 μm or smaller pore size polycarbonate screen-type filters.

Based on this literature review, it appears that the current definition of dissolved metals as the fraction that passes through a 0.45 μm pore size filter is inadequate. Studies have shown that substantial amounts of colloidal material can pass through this pore size, and these colloids can accumulate large amounts of trace metals. As a result, the dissolved fraction can be mistakenly viewed as contaminated with metals when in fact it is colloidal bound metals which are measured. Unfortunately, there appears to be no information on how colloidal bound metals can affect bioavailability and/or toxicity of metals to aquatic organisms. Therefore, an important avenue for future research is to evaluate the toxicity of these colloidal bound metals.

All parties involved in monitoring metals in Canadian receiving environments should be updated on some of the potential contamination problems associated with processing water samples for metals analysis. It is quite possible that some mine personnel responsible for collecting routine monitoring samples are unaware that sample contamination of the water can be a major problem. We caution against making immediate changes to the current protocol of filtering water through a 0.45 μm filter to collect the dissolved metal fraction. Before this is attempted, substantial research is needed to determine methods that best define the dissolved fraction. However, there are a few specific recommendations that can be made at this time:

- We recommend that all agency, industry and research biologists involved in AETE activities use the same filters (e.g., pore size, manufacturer), filter the same amount of water, collect an initial aliquot to quantify artifact-affected constituents (Horowitz et al., 1996), use the same filter pump, clean materials in a similar manner between sample processing, and take detailed records on field techniques and sample processing. In other words, it is important that a sampling protocol for collection of water samples for analysis of total and dissolved metals be designed and that this protocol be used by all parties (e.g., mine personnel, government scientists). Standardizing filtration procedures will potentially ensure that water quality data is similar within the mining industry and, thus, improve the quality of data.

- We recommend that recent papers in *Environmental Science and Technology* (Taylor and Shiller, 1995; Horowitz et al., 1996;) and the USEPA=s Method 1669:Sampling ambient water for trace metals at EPA water quality criteria levels (U.S. EPA, 1995), serve as a technical basis to develop a Auser friendly@ training workshop on contamination problems in mine monitoring. This training material (e.g., video, overheads, field visits) would emphasize clean techniques and the need for standardization of sample collection procedures.

- As was stated previously, research is needed to evaluate different methods of collecting and processing dissolved metals. This would entail testing different filter types, filter methods, etc. in order to collect water samples that are most representative of metals in the dissolved state.

- In conjunction with the above recommendation, we suggest that a technical workshop be convened which includes aquatic ecotoxicologists, biogeochemists, inorganic chemists, and regulators to discuss the adequacy of the current definition of dissolved metals and potentially develop a targeted investigation program.

- Conduct toxicity and bioassay experiments to determine the biological effects of metals in the colloidal fraction on aquatic organisms.

In summary, we believe the question of what constitutes the dissolved metal fraction is an important issue for metal monitoring in Canada. If aquatic effects monitoring included dissolved metals, some mines might exceed acceptable limits for dissolved metals due to inadequate filtration techniques and because metals bound to colloids pass through the filter into the filtrate. The implications of this are obvious and should be addressed.

1.2 Factors that Affect Metal Speciation and Toxicity

An aquatic organism is exposed to metals in both food (particulates) and solution (dissolved and potentially on colloids). Within each phase, the organism is exposed to a variety of physicochemical forms of each metal, and each form differs in its accessibility to the organism (Luoma, 1983). In general, it is thought that it is metals in the dissolved fraction, particularly the free metal ion, that are the most bioavailable species to aquatic organisms. There is much qualitative evidence to support this notion, especially in defined, synthetic media (Campbell, 1995). However, this relationship appears to break down in natural waters, particularly in the presence of natural dissolved organic matter (Campbell, 1995).

Metal uptake by aquatic organisms is controlled by environmental conditions at the biological membrane. Processes that control metal uptake include:

- Characteristics of the membrane itself
- Reactivity of each metal form with the biological membrane
- Presence of other metals or major cations which may antagonize or stimulate metal uptake
- Factors such as temperature which affect uptake rates
- Ecological characteristics of the organism.

This section reviews some of the factors that affect toxicity of metals to aquatic organisms to determine what metal species are the most bioavailable. Specifically, we address the issue of whether the dissolved or total metal fraction best explains toxic effects. It is important to point out that the identity of metal forms binding to a biological membrane is biologically unimportant, as no single metal species is more or less available than another (Campbell, 1995). The free metal ion activity reflects the chemical reactivity of the metal. This reactivity influences the metal=s reactions at the cell surface, and hence its

bioavailability (Campbell, 1995). Furthermore, this review primarily relates to metals that form dissolved cations in natural waters (e.g., Al, Cd, Cu, Zn).

We also review various tools, such as chemical speciation models, that may be of use to predict metal speciation in aquatic ecosystems. Some of these tools may be of immediate use in monitoring metals in Canadian freshwater ecosystems, while others show promise with further development.

1.2.1 Hardness and Alkalinity

Water hardness and alkalinity are thought to be important properties governing the toxicity of metals. In natural waters, hardness is predominantly determined by calcium (Ca^{2+}) and magnesium (Mg^{2+}) cations, while alkalinity is controlled by the bicarbonate system (i.e., HCO- and CO_3^{-2}) (Davies and Brinkman, 1993). Alkalinity and hardness are both expressed as calcium carbonate ($CaCO_3$), hence their implied relationship in natural waters. However, in some wat s there can be a large difference between the two (e.g., low alkalinity can coincide with high hardness).

Hardness does not play a role in controlling speciation of metals in water, but it does influence metal toxicity through antagonistic mechanisms. Specifically, Ca^{2+} and Mg^{2+} ions will compete with free metal ions for binding sites on gill surfaces (Davies and Brinkman, 1993).

Alkalinity has been proposed as more important in determining metal toxicity than hardness (Davies and Brinkman, 1993). Research has shown that the Cu^{2+} and Cu-hydroxy complexes are the chemical species most toxic to fish and that alkalinity is more important than hardness in controlling Cu^{2+} concentration (Pagenkopf et al., 1974; Erickson et al., 1996). Thus, it appears that alkalinity may be more important in controlling metal toxicity than water hardness, and that in the case of Cu, the Cu^{2+} and the hydroxy ions are the most toxic and their concentrations are somewhat controlled by alkalinity.

1.2.2 pH

The pH of water has a major influence on the physical and chemical forms of metals and metal compounds in the aquatic environment because it controls the solubility and concentrations of major metal species (Campbell and Stokes, 1985). Increasing the acidity of a solution increases the concentrations of free metal ion in that solution. This is due to the competition between H^+ and metal ions for binding sites on inorganic and organic ligands. Because of the relationship between pH and concentration of free metal ions, it has been assumed that metals are more likely to be toxic to biota in acidic than in neutral waters (Campbell and Stokes, 1985). However, more recent research suggests that a decrease in pH can result in a decreased biological response (e.g., toxicity) for some

metals (Cd, Cu, and Zn). The mechanism proposed to explain this relationship is that a decrease in pH leads to an increase in the concentration of H^+ ions in solution, and that these ions can compete for binding sites on cell surfaces with free metal ions. For those organisms that are sensitive to pH values <6, this interaction is of academic interest as the lower pH will cause negative biological effects (Campbell and Stokes, 1985).

Evidence to support the notion that H^+ ions compete with metal ions for binding sites was provided by Hare and Tessier (1996). This study showed that uptake of Cd by the insect larva, *Chaoborus punctipennis*, was best described by free-ion activity, provided that competition for biological uptake sites between H^+ and Cd^{+2} was considered. Using larval fathead minnow (*Pimephales promelas*), a linear relationship was established for pH and 96 hour LC50s based on total Cu (dissolved Cu was not reported) (Welsh et al., 1993). It was hypothesized that Cu toxicity increased with a decrease in pH, because the amount of free Cu ion bound to organic carbon would decrease in response to an increase in H^+, as the hydrogen ion would compete with Cu for binding sites on the carbon molecule. Due to this competitive relationship, there would be greater amounts of free Cu ion in solution that could bind to gill surfaces causing mortality. An increase in H^+ also led to higher mortality to embryos and larvae of the South African clawed frog, *Xenopus laevis*, in a modified frog embryo teratogenesis assay. This result was most likely a result of the higher Cu^{+2} activity (Buchwalter et al., 1996), which was associated with a lower pH.

In contrast, an increase in pH can also affect metal speciation and toxicity. Meador (1991) proposed that as pH increases, the amount of copper hydroxides increases, and it is possible that one or several of these hydroxide complexes contributed to the toxic response exhibited by *Daphnia magna*. It was suggested that Cu^{+2} may be the predominant species causing short term toxicity, but it may not accurately reflect long term toxicity because chronic effects may be due to other copper complexes (e.g., copper hydroxides and organic complexes).

These results suggest that changes in pH can affect metal speciation and toxicity in complex ways, and that metal species other than the free metal ion can be toxic. The effect of pH on metal speciation is metal specific and some of the mechanisms involved are unknown or appear contradictory.

1.2.3 Suspended Particulate Matter and Dissolved Organic Carbon

The concentration, size class, and organic content of particulates play a major role in determining the amount of metal complexed to solid material, and therefore the availability of that metal to aquatic organisms. For example, naturally occurring dissolved organic compounds such as humic acids have a substantial effect on the bioavailability of metals (Winner, 1984; Meador, 1991; Welsh et al., 1993). In some cases, toxicity on the basis of free metal ion has been reported to be roughly constant as organic matter changes, leading

to the conclusion that organic-complexed metals are essentially non-toxic (Winner, 1985; Meador, 1991; Erickson et al., 1996; Hare and Tessier, 1996). For example, Welsh et al. (1993) showed a linear relationship between log toxicity of total Cu and log dissolved organic carbon (DOC) concentration that explained 92% of the variation in the model. Hare and Tessier (1996) showed that the unexplained variability of Cd concentrations in the insect larva *Chaoborus* was reduced by more than half when Cd complexation by dissolved organic matter was taken into account.

In general, suspended inorganic and organic matter will bind metals rendering them less bioavailable to aquatic biota; however, some of these complexes may also exert a toxic effect (Campbell, 1995). For instance, higher concentrations of dissolved organic carbon increased the toxicity of the free Cu ion to *Daphnia magna* (Erickson et al., 1996). Winner (1984) found that adding humic acid (i.e., DOC) increased the acute and chronic toxicity of cadmium to *Daphnia magna*. Similarly, Giesy et al. (1977) observed that adding the smallest molecular weight organic fractions (i.e., <0.0009 μm) to a test solution increased Cd toxicity to *Simocephalus serrulatus* (Daphnidae), although this small size fraction also exhibited the greatest binding capacity. Under certain water quality conditions, the addition of fulvic acid was shown to enhance toxicity of Cu to the frog *Xenopus laevis* (Buchwalter et al., 1996). At the moment, there is no clear understanding of how these organo-metal complexes elicit a negative response in aquatic organisms. Thus, it seems that dissolved organic matter cannot be treated as a simple hydrophilic ligand that will bind metals thereby decreasing their bioavailability (Campbell, 1995). In contrast, dissolved organic matter is multifunctional, and it appears that some metal-organic matter complexes may be bioavailable to an organism and contribute to toxicity.

It is important to note that metals are naturally present in the Earth=s crust and concentrations of some elements may be quite high, especially in metal-rich soils. Thus, metal concentrations in streams and lakes in these mineralized areas have the potential to be high and even exceed water quality criteria. Specifically, the natural metal content of suspended soil particles can influence total metal levels in water. For example, copper and zinc levels in the Earth=s crust have been measured at 55 mg/kg and 70 mg/kg respectively (Demayo, 1992), and these concentrations correspond to 5 ug/l and 7 ug/l of copper and zinc in the non-filterable residue or suspended particulate matter. Such a phenomenon highlights the need to adequately filter water samples and to use dissolved metals analysis whenever large amounts of suspended solids are present.

1.2.4 Biotic Factors Affecting Uptake of Metals

Food is potentially an important vector of metal uptake to aquatic organisms (Luoma, 1983); however, establishing the relative importance of food versus water as routes of metal exposure has proven to be difficult. Experiments using Pb showed that uptake from food and freshwater did not exceed uptake from solution alone (Hodson and Sprib, 1978

from Luoma, 1983). In contrast, Young (1975 from Luoma, 1983) observed that food was an important source of Zn uptake by snails (*Littorina obtusata*). In an attempt to develop a model for determining the importance of biotic uptake in causing toxicity, Dallinger et al. (1987) proposed the "food chain effect." This model hypothesizes that metal transfer through aquatic food chains can be high enough to be harmful to fish under certain ecological situations. The findings that the bioconcentration of metals decreases moving up the aquatic food chain from algae to fish should not obscure the fact that transfer of metals through food chains remains an important issue in metal assimilation by fish. Most heavy metals are effective at low concentrations, so even low assimilation rates are sufficient to attain biologically significant or harmful concentrations in tissues (Jackim et al., 1970; Eisler, 1988). Furthermore, metal contamination in aquatic ecosystems is more often reflected by high metal levels in sediments, macrophytes and benthic animals than by elevated concentrations in water (Prosi, 1981). Depending on their habitat preferences or specialized food requirements, fish may be endangered in such situations, particularly bottom-dwelling species which can accumulate high metal levels because of their ingestion of metal-loaded sediment and benthic invertebrates (Ney and Van Hassel, 1983). For example, survival of rainbow trout (*Onchorhynchus mykiss*) fed with benthic invertebrates containing elevated metals from Clark Fork River, MT, USA and exposed to metals in water was significantly lower than trout fed with invertebrates from a control stream and also exposed to metals in water (Woodward et al., 1994). The authors concluded that Clark Fork River invertebrates were the major source of metals that accumulated in rainbow trout in their study.

Metal availability also differs among functional feeding groups (Luoma, 1983; Kiffney and Clements, 1993). Benthic invertebrates that feed primarily on periphyton (i.e., algae, bacteria, fine detritus and sediment accumulating on rock surfaces) bioaccumulated significantly more metals (Cd, Cu, Zn) than predaceous invertebrates (Kiffney and Clements, 1993). In contrast, higher concentrations of Hg were measured in predatory sharks than in sharks that are benthic feeders. This result may reflect a greater availability of Hg from fish tissue than from benthic organisms, however, higher concentrations of methylmercury in fish tissue may also influence these differences (Luoma, 1983). The strength of metal binding to particulates may also determine metal bioavailability in an organism=s gut. Luoma and Jenne (1976, 1977) found that uptake of Zn bound to biogenic carbonates and organic detritus was greater than from Fe or Mn oxides. The differences in metal availability were inversely related to the strength of the metal binding to particulates. Specifically, sediments that exhibited the greatest metal binding affinity were substrates from which metal bioavailability was lowest.

Although metals adsorbed and/or absorbed by particles may not be as directly available to aquatic organisms as free metal ions, these metal-coated particles are indirectly available through biotic (e.g., ingestion) and physical processes (e.g., adsorption). As was

mentioned previously, uptake of metals by aquatic organisms through feeding may contribute to toxicity, especially over the long-term.

1.3 Influence of the receiving environment and metalspeciation

Another potential problem with relying on dissolved metals to predict biological effects involves metal speciation in effluents, and the potential transformations that may occur in moving from the chemical environment of effluent to the chemical environment of receiving water. For example, experimental studies have shown that Al toxicity in dynamic systems cannot be derived from the total concentration of the metal only (Witters et al., 1996). In this study, brown trout (*Salmo trutta* L.) experienced acute mortality (98% in 48 hr) in neutral water mixed with acidic, Al-rich water, even though this mixture contained low Al concentrations (185 ug/L) and had a pH of 6.4 (Witters et al., 1996). In contrast, mortality was lower when fish were exposed to higher Al levels (445 ug/L) and pH was lower (4.6). Thus, it is evident from this study that Al toxicity to fish is not related to the total Al concentration in the water. Based on these results, changes in chemical species and toxicity can occur when waters of different physicochemical quality (pH, temperature, hardness, alkalinity, DOC, suspended solids) meet at the confluence of rivers or when wastewater is discharged into receiving waters.

In summary, there are many environmental factors (reviewed in Section 2.2) that can influence metal forms and the potency of these forms to aquatic organisms. We hypothesize that along a gradient (e.g., oligotrophic to eutrophic) of water quality conditions, the free metal ion will vary in its relative toxicological importance. For example, we hypothesize that in neutral, oligotrophic waters, the free metal ion will be the most bioavailable and toxic form. As ecosystem productivity increases, other metal-complexes, such as small molecular weight organo-complexes, may contribute to toxicity as well (Campbell, 1995). Below, we present one approach AETE may take to evaluate, on a site-specific basis, whether metals in the dissolved fraction explain most of the toxicity to aquatic organisms.

- Select mine sites that discharge into receiving environments representative of a given level of ecosystem productivity (i.e., oligotrophic to eutrophic). This productivity gradient could be defined by levels of primary productivity, inorganic nutrients, etc. For example, Buttle Lake, Vancouver Island, B.C. which receives mining discharge from Myra Creek would represent an oligotrophic system, while the St. Mary River downstream from the Sullivan Mine discharge near Kimberly, B.C. would represent a more productive system.

- At these sites, collect water downstream from the mining discharge; compare results from toxicity experiments using water filtered through a 0.45 μm filter and unfiltered water. If, for example, mortality is 50% using unfiltered water versus 30% for filtered water, this result would suggest there are particulate bound metals that contribute to toxicity.

- Further fractionation of this water, followed by toxicity experiments, would provide information on what size fractions are causing toxicity.

In summary, we recommend that research be funded that investigates the relationship between filter pore size and toxicity to aquatic organisms. In these experiments, receiving water downstream from a mine would be filtered through a series of filters, such as 0.45, 0.3, 0.2, 0.1, and a 0.05 μm filters. Results from such a study would provide data on the correlation of toxicity with pore size. We are unaware of other research that has addressed the relationship between filter pore size and metal toxicity. Furthermore, it is difficult to predict how results will influence costs of aquatic effects programs; however, it is important to understand mechanisms of metal toxicity in natural waters. With this knowledge, it may be possible to develop treatment strategies that will minimize the effects of mine effluent on the receiving environment, which in the long run may decrease costs. Moreover, such studies would provide some insight into whether the total or dissolved fraction best explains toxicity on a site-specific basis and thus could be incorporated into a monitoring program.

1.4 models to predict metalspeciation

Currently, there are a number of models that are used to predict metal speciation in freshwater environments. Some of these models, such as metal translators, are very simple empirical approaches that convert a total recoverable metal concentration to a dissolved fraction (i.e., water passed through a 0.45 μm filter). This approach is based on the assumption that the dissolved metal more closely approximates the bioavailable fraction of metal in the water column. The U.S. Environmental Protection Agency (U.S. EPA) has adopted these metal translators on a site-specific basis. An obvious implication of using dissolved metals in regulatory permits is that it would allow dischargers to monitor only this fraction as opposed to both total and dissolved. This would substantially lower analytical costs.

Other potential monitoring tools include chemical equilibrium models, and an extension of these models, the fish gill model (Janes and Playle, 1995). In the following sections, we review different approaches to understanding chemical speciation of metals in the receiving environment and how these various approaches may be of use to those involved in mine monitoring in Canada.

1.4.1 Metal Translators and Conversion Factors

The U.S. EPA issued a policy memorandum on October 1, 1993, titled "Office of Water Policy and Technical Guidance on Interpretation of Aquatic Life Metals Criteria". This document states that "the use of dissolved metal to set and measure compliance with water quality standards is the recommended approach, because dissolved metal more closely approximates the bioavailable fraction of metal in the water column than does the total recoverable metal." However, because Section 304(a) of the Clean Water Act requires that criteria be expressed as total recoverable metal, application of a conversion factor or translator is needed to convert dissolved metal levels to total recoverable.

Based on the premise that the dissolved fraction better represents the biologically available metal than does total recoverable, these conversion factors have the effect of reducing the water quality criteria concentrations (U.S.EPA, 1996). The conversion factors are predictions of how different the criteria would be if they had been based on measurements of the dissolved concentrations in the toxicity tests that were most important in the derivation of the criteria. Therefore, each metal's total recoverable criterion must be multiplied by a conversion factor to obtain a dissolved criterion that should not be exceeded in the water column. For example, the silver (Ag) acute conversion factor of 0.85 is a weighted average and is used as a prediction of how much the final acute value would change if dissolved silver had been measured. At a hardness of 100 mg/L as calcium carbonate ($CaCO_3$), the acute total recoverable criterion for Ag is 4.06 μg/L while the dissolved criterion is 3.45 μg/L.

When calculating a conversion factor for a dissolved metal ambient water quality criterion to a total recoverable concentration in the effluent, it is most appropriate to analyze for total and dissolved metal concentrations in the mixture. This ratio of dissolved to total recoverable metal levels can then be used to translate from a dissolved concentration in the water column downstream of the effluent discharge (the criterion concentration) to the total recoverable metals in the effluent that will not exceed that dissolved concentration in the water column (U.S. EPA, 1996).

To develop translators for site specific conditions, it is necessary to conduct a field sampling program using appropriate clean analytical techniques. A key factor in metals bioavailability to biota in the water column is the partitioning of metals between the solid phase material and water (U.S. EPA, 1996). Total suspended solids (which contain dissolved and particulate organic material and inorganic minerals [e.g., clay particles]) is one of the most important physicochemical parameter to measure, as well as pH, alkalinity, hardness, discharge volume, and temperature. All of these environmental variables can vary spatially and temporally; therefore, it is important to consider the normal range of chemical conditions when designing a field program to develop a site-

specific metal translator. The field program should generate data on total recoverable, dissolved, and particulate metal fractions; total suspended solids (TSS); particulate organic carbon; pH; hardness; and stream (volume) flow. Furthermore, a sampling program should be designed that allows for enough statistical power (i.e., the ability to detect a significant difference when such exists) to determine the mean concentrations of total and dissolved metal within an acceptable confidence interval of the true mean, and to determine means precisely enough so that the mean total concentration is significantly different from mean dissolved concentration. If data exhibit these differences, one can calculate the site specific metal translators based on the dissolved-to-total metals ratios.

There are a number of problems associated with this approach (U. S. EPA, 1996). Probably most important is the validity of the assumption that the dissolved metal fraction explains toxicity at each and every site. We suggest that this assumption should be treated as a research hypothesis that should be tested with controlled experiments. Additional problems will arise when insufficient information is available when developing a site-specific translator, and generic information is applied from other sites. This approach introduces considerable uncertainty into the evaluation; we recommend that every effort be made to collect the necessary water quality information for developing site-specific translators. In addition, translators do not consider bioaccumulation of metals, which may be problematic in that some benthic organisms filter particulate material from the water column. Thus, we suggest that site-specific translators are an approach worth considering, but there needs to be a more rigorous evaluation of this method. Such research is presently being conducted by the U.S. EPA and the AETE should track findings carefully.

1.4.2 Chemical Speciation Models

In this section, we review equilibrium modeling of dissolved metals in freshwater ecosystems. It is important to point out that these models do not predict metal bioaccumulation or toxicity to biota. Furthermore, if the output of these models is used to predict the uptake or toxicity of a metal ion, rapid, dynamic equilibrium must be assumed.

Some of the current speciation models include MINTEQA2 (Allison et al., 1991), MINEQL$^+$ (Schecher and McAvoy, 1992) and WHAM (Kramer et al, 1996; Hare and Tessier, 1996). In general, these models all share common elements (Kramer et al., 1996) and the output they produce depends on the quality of the thermodynamic database. MINTEQA2 and MINEQL$^+$ contain extensive databases for inorganic solutes and solids, while MINTEQA2 also has databases for simple organic ligands and a database for dissolved organic matter; however, the simple organic ligand database is considered flawed (Kramer et al., 1996). The WHAM model contains data on the metal binding of 30 metal ions, using over 80 published data sets for a variety of soil fulvic acids and dissolved organic matter samples, over a pH range of 2- 9. This model was successfully used to improve prediction of the empirical relationship between Cd concentrations in *Chaoborus*

punctipennis and free ion Cd concentration in water (Hare and Tessier, 1996). Unexplained variability of [Cd] in *Chaoborus* was decreased by half when dissolved organic matter was taken into account using the WHAM model.

To model the dynamics of metals in aquatic systems, the minimum list of variables needed is pH, alkalinity, hardness (preferably Ca ion concentration), ionic strength, total dissolved solids (TDS) (specific conductivity potentially can be used as a surrogate for ionic strength and TDS), DOC, total dissolved metal, unique anthropogenic inputs (e.g., [EDTA]), and preferably Al and Fe (III) (Kramer et al, 1996). Speciation models can make reasonable estimates of metal speciation in toxic waters, but there are three potential sources of error that must be considered when applying these models.

1. Care must be taken when extrapolating model output to situations which have not been sufficiently parameterized (Kramer et al., 1996). For example, laboratory water chemistry data on which these models are based do not represent pH conditions higher than 7 very well.

2. Bad data can affect model predictions, and the extent of this variation can differ from one water quality variable to another (Kramer et al., 1996).

3. Parameter uncertainty can also affect quality of model output. For example, WHAM uses the average value for pK_{MH} (negative logarithm of the intrinsic equilibrium constant for metal-proton exchange), which is the key parameter with respect to metal binding. Typically, the range of values differs by ± 0.2 log units, which can affect the predicted concentration of free metal ions by an order of magnitude in some cases (e.g., Cu) (Kramer et al, 1996).

An important question regarding chemical speciation models is how good are they at predicting toxicity of a discharge to aquatic organisms? These models are equilibrium models, and can provide data on what fraction or species of a metal would be toxic or bioavailable. However, once a free metal ion is removed from a system by an organism, compensatory dissociation of complexes must occur to reestablish equilibrium. Thus, chemical models indicate the potential of a metal to be toxic.

We suggest that these models should be used in conjunction with toxicity testing of receiving waters. Specifically, water quality data from a mine site could be collected and used in one of the above models. The model output would provide predictions as to the forms and concentrations of metal-complexes in the site water, which could then be used to predict the toxicity of the water. Such an approach is necessary to improve the applicability of these models and their use in the regulatory arena.

1.4.3 Fish Gill Models

An extension of the chemical equilibrium models was recently developed, including an additional organic receptor that interacts with metals in solution, the fish gill (Janes and Playle, 1995). Gills of freshwater fish have two important physiological functions: (1) they are the site of gas transfer (e.g., O_2 and CO_2), and (2) they are the site of active ion uptake (e.g., Na and Cl) to counter diffusive loss of ions down the electrochemical gradient from fish to water. Thus, at its simplest level, it is possible to formulate an equilibrium model of toxicity by including the gill receptor as a ligand (Janes and Playle, 1995). For example, using a synthetic softwater medium, rainbow trout (*Oncorhynchus mykiss*) were exposed to Ag, while cations (Ca, Na, H^+) and complexing agents (DOC, Cl, S_2O_3) were added to keep Ag off the gills and to calculate conditional equilibrium binding constants at the gills. All experimentally determined equilibrium binding constants were entered into the MINEQL$^+$ model to predict Ag binding at trout gills. Thus, the model considered complexation of Ag in the water surrounding a fish and cation competition for Ag binding sites on the gills. For a series of natural waters, model-predicted gill Ag concentrations correlated well with observed gill concentrations (Janes and Playle, 1995). Therefore, this study inserted a biological component into a powerful aquatic chemistry program.
This approach may ultimately allow better understanding and prediction of interactions of metals such as Ag with sensitive biological membranes. What is needed in the future is to develop metal-gill interaction models to predict acute toxicities while correlating gill metal concentrations with observed mortalities. In addition, similar models need to be developed for invertebrates.

1.5 Summary of technical evaluation

The current definition of dissolved metals as the metal component of a water sample passing through a 0.45 μm filter appears inadequate. We recommend research be conducted to test the relationship between filter pore size and toxicity. Furthermore, we suggest that a forum be held that evaluates techniques that can be used as standard protocols for the processing of water samples in mine monitoring. Material from this forum can then be used to train personnel involved in aquatic effects monitoring as to the most efficient approach to sample collection. We also suggest that regulatory personnel, consultants, and research scientists consider alternatives to the simple dichotomy of dissolved vs. total metals. This is especially true for systems with complex water chemistry, such as estuaries and ecosystems receiving multiple discharges. However, it is important to note that, while an alternative definition may better represent the dissolved fraction, it may be more time consuming and expensive to measure. In addition, there may be no measurable change in correlation to toxicity.

Based on review of the literature, the concentration of free ion of at least some metals (i.e., Cd, Cu, Fe, Mn, and Zn) is the chemical species that is bioavailable to aquatic

organisms. Nevertheless, we suggest that this relationship should be evaluated on site-specific basis, because of the inherent natural variability in water chemistry from site to site. For example, we recommend that toxicity studies using filtered and unfiltered water be conducted at Canadian mine sites along a productivity gradient (i.e., oligotrophic to eutrophic). These experiments may provide insights into what fraction (total vs. dissolved) best explains toxicity and how this pattern changes with nutrient condition.

We recommend the use of chemical speciation models to provide information on the metal chemistry in receiving environments. With output on metal speciation from these models, it would be then be possible to predict the potential toxicity of the metal fractions in water based on a literature review. These predictions then should be validated with toxicity experiments. These data would provide insights into whether or not it is necessary to monitor total and dissolved metals, or whether dissolved metal levels provide enough information for a regulatory agency to evaluate compliance with water quality regulations.

Finally, it is important to follow the use of conversion factors and metal translators currently being adopted by the U.S. EPA, because these approaches may be useful to the Canadian monitoring community. However, before Canada incorporates such approaches, we suggest that the use of conversion factors/metal translators be examined using toxicity studies to verify their usefulness.

3.0
ANALYTICAL TECHNOLOGIES FOR METALS ANALYSIS

In the past, metals analysis of natural waters has focused on total and dissolved metals to provide an indication of metal bioavailability. Recently, and as discussed in Section 2.2, it has been realized that the toxicity of metals to aquatic organisms may be more a function of the concentration of free metal ion rather than total or dissolved metal concentration (Sunda and Guillard, 1976; Anderson and Morel, 1978, 1982). The following sections discuss and compare various analytical techniques for metals detection. The methods discussed include: (1) established techniques that have been used to measure total and/or dissolved metals; and (2) new technologies that aim to measure free metal ion concentrations. Some examples of analytical method detection limits are provided in Table 3-1. In addition, we provide (Table 3-2) cost estimates from several analytical laboratories. These are 1996 prices and subject to change. Instrumental detection limits were not provided as they do not reflect achievable laboratory detection limits used in routine water quality monitoring.

3.1 ESTABLISHED TECHNOLOGIES FOR METALS ANALYSIS

Currently, metal analysis focuses on the total and/or dissolved metals fraction. The techniques described below measure the total metal concentration. The fraction of metal which is measured is dependent on sample treatment prior to analysis. For example, in dissolved metals analysis, the sample is passed through a 0.45 μm filter and fixed to a pH < 2 with a preservative such as nitric acid.

Induced Coupled Plasma (ICP)

ICP techniques are the most commonly used methods for metal detection. These techniques include: induced coupled plasma-mass spectrometry (ICP-MS); induced coupled plasma-optical emission spectrometry (ICP-OES); and induced coupled plasma atomic emission spectrometry (ICP-AES). These techniques are U.S. EPA and B.C. Ministry of Environment, Lands, and Parks (BCMELP) approved (BCMELP, 1994; Mach et al., 1996) and they determine total metal content unless physical or chemical separation steps are performed prior to analysis. ICP technologies have made great strides in the last 20 years, as practicality and cost-effectiveness of the instrumentation has increased. The cost of using these instruments remains higher than for Atomic Absorption Spectrometry (AAS) instruments (see next section) of comparable analytical sensitivity (Jacobs, 1996), but the ability of ICP to scan multiple elements makes it more cost effective than AAS. In addition, ICP has fewer chemical interferences than does AAS due to the extremely high

plasma temperature achieved by ICP; however, physical differences in the sample matrix and spectral interference are more likely to be of concern for ICP techniques (Robin 1988; Jacobs 1996).

Table 4-1. Method detection limits (MDL) for some existing analytical technologies (ug/L).

Element	FAAS[1]	HGAAS[1]	GFAAS[1]	CVAAS[1]	ICP-MS[1]	ICP-OES[2]	ISE[3]	ASV[4]
Aluminum	50	-	5	-	1	100	-	-
Antimony	-	2	1	-	0.1	100	-	-
Arsenic		2	1	-	1	100	-	-
Cadmium	2	-	0.2	-	0.05	5	10	<0.005
Chromium	20	-	1	-	0.05	7.5	-	-
Copper	10	-	1	-	0.02	5	60	<0.005
Iron	20	-	5	-	6	15	-	-
Lead	30	-	1	-	0.05	25	200	<0.005
Manganese	10	-	1	-	0.05	2.5	-	-
Mercury	-	-	-	0.05	-	-	-	-
Molybdenum	-	-	1	-	0.1	15	-	-
Nickel	20	-	1	-	0.2	100	-	-
Selenium	-	2	1	-	1	100	-	-
Silver	-	-	0.1	-	0.05	7.5	20	-
Zinc	10	-	0.5	-	0.2	25	-	<0.005

1 Metal detection limits (MDL) are based on detection limits reported by CAEAL (C anadian Association for Environmental Laboratories) (McQuaker, 1997). The method detection limits (with the exception of mercury) are for dissolved metals. See Section 4. 1 for definition of MDL.

2 Data for ICP-OES from ASL Analytical Laboratories, Vancouv er, B.C.

3 Data for ISE from Radiometer, Copenhagen.

4 Data for ASV technologies from Deaver and Rodgers, 1996.

Table 4-2. Summary of technologies for metals analysis.

	METAL FRACTION ANALYZED	COST[1]	COMMERCIAL AVAILABILITY[2]	EASE OF USE[2]	SCIENTIFICALLY ACCEPTED[3]
Established					
Graphite Furnace Atomic Absorption Spectrometry (GFAAS)	total/dissolved	$10-12/metal	H	H	US-EPA/BCMELP
Hydride Generation Atomic Absorption Spectrometry (HGAAS)	total/dissolved	$12-15/metal	H	H	US-EPA/BCMELP
Flame Atomic Absorption Spectrometry (FAAS)	total/dissolved	$8/metal	H	H	US-EPA/BCMELP
Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)	total/dissolved	$35-50/multi-metal scan	H	H	US-EPA/BCMELP
Inductively Coupled Plasma Mass Spectrometry (ICP-MS)	total/dissolved	$90-125/multi-metal scan $30/metal	H	H	US-EPA/BCMELP
Alternative					
Anodic Stripping Voltammetry	free ion	no data	L	L	N/A
Ion Selective Electrodes	free ion	no data	L	L	N/A
Ion Chromatography/AAS	free ion	$250/metal	L	L	N/A
Bioassay Data	free ion	no data	L	L	N/A

1 Cost ranges represent prices from several analytical laboratories.
2 H = High ; L = Low (these ratings are relative).
3 N/A = Technologies which have not been endorsed by any government agency.

The first commercial ICP-MS was introduced in 1983 by Sciex, Canada, and is now one of the most widely used ICP techniques. Its advantages include: superior sensitivity; simple spectra; and the ability to obtain isotopic information. For example, only Graphite Furnace - AAS has similar analytical sensitivity as ICP-MS (Hall, 1992). ICP-MS is also ideal for water analysis and selective leaches. As with every analytical technique, there are disadvantages to using ICP-MS, such as significant matrix effects. Specifically, the number of analyte ions reaching the detector can be altered by the effects of concomitant species in solution, resulting in suppression or enhancement (Hall, 1992).

A key difference between ICP-MS and ICP-AES is that in ICP-MS, sample ions must be physically transported from the plasma to the mass spectrometer whereas in AES, photons are passively observed. This interface region continues to be problematic in analysis by ICP-MS (Hall, 1992). In addition, ICP-AES is more tolerant of high salt levels than ICP-MS.

Detection limits of ICP-OES are higher than both ICP-MS and AAS techniques (Table 3-1), but sample costs for multi-element scans with ICP-OES are considerably lower than for ICP-MS (Table 3-2). Currently, cost per sample for ICP analysis ranges from $90 to $125 dollars per metal scan, depending on the type of technique used and metals involved (Table 3-2).

Atomic Absorption Spectrometry (AAS)

In the past, AAS techniques were some of the more commonly used methods for metal detection in water samples. These techniques include: Flame-AAS (FAAS), Graphite Furnace-AAS (GFAAS), Hydride Generation-AAS (HGAAS) and Cold- Vapour-AAS (CVAAS). In theory, these instruments have the ability to perform simultaneous metals analysis, but usually they can only perform sequential multi-element analysis (Jacobs, 1996). Thus scanning for a wide range of metals in a water sample is time consuming and expensive. AAS techniques are U.S. EPA and BCMELP approved (Mach et al., 1996; BCMELP, 1994).

The main limitations of AAS techniques stem from detection limits and sensitivity of a particular analyte (Rubeska, 1988). AAS techniques suffer from significant interferences due to the sample matrix and from physical and chemical conditions encountered in the light path. Interferences include background absorption (nonatomic), vapourization, ionization, and spectral. Most interferences can be overcome or minimized through techniques such as matrix matching and background correction techniques (Fresenius et al., 1988).

AAS techniques differ in the type of sample preparation and atomization. For example, only a few elements can be practically determined using HGAAS, as this technology depends on the ability of the element to form volatile hydrides (Hg, As, Bi, Sb, Se, Te) or high volatility. For these few elements, HGAAS has the greatest sensitivity of AAS techniques (Jacobs, 1996).
GFAAS has the lowest detection limits (Jacobs, 1996); however, it also has the slowest sample throughput. Some of the limitations of GFAAS include: interference from non-specific background absorption, which is a much more severe problem than in FAAS and vapour phase interference by other metal species (Rubeska, 1988; Fresenius et al., 1988).

1.6 Alternative Technologies for Metals Analysis

This section describes analytical techniques that are less common than ICP and AAS. These alternative technologies focus on measuring free metal ion concentrations in natural waters, either by direct probe or by physical or chemical separation of the water sample.

Anodic Stripping Voltammetry (ASV)

ASV has proven to be reasonably successful at determining levels of uncomplexed metals in water (Davies and Brinkman, 1993). Differential pulse anodic stripping voltammetry (DPASV) is an ASV technique that has been employed with reasonable success and can measure concentrations of metals in solution as low as 0.005 μg/L (Deaver and Rodgers, 1996). Deaver and Rodgers (1996) found that DPASV measured Cu concentrations and provided a better estimate of Cu toxicity to *Hyalella azteca* than AAS techniques measuring total Cu. This technique is highly sensitive and can be employed to determine metal levels; however, it is not applicable to all metals (Davies, 1986). Metals that can currently be analyzed with this technique include Cu, Pb, Cd, Zn, bismuth (Bi), Sb and thallium (Tl) (Lund, 1986).

The ASV technique measures labile metal forms (easily reducible metal complexes) in addition to the free metal ion (Florence et al., 1983), but it cannot distinguish between free metal ions and labile complexes that disassociate or are directly reduced at the electrode surface (Sunda and Hanson, 1987). For example, the free Cu^{2+} ion concentration is obtained by correcting for the concentration of metal-inorganic and labile, metal-organic species, provided that the concentration of each of these other electroactive species can be measured. This is possible with the inorganic and well-defined organic ligands (Hart, 1981), but other metal complexes will disassociate during the period of metal accumulation at the electrode and, as a result, contribute to the stripping current which causes an overestimation of the free Cu^{2+} ion concentration (Hart, 1981). Furthermore,

organic matter can adsorb on the electrode and depress the signal without complexation taking place (Nordstrom, 1996).

With current technology, ASV is a very time consuming (up to 20 minutes per sample) (Deaver and Rodgers, 1996) and requires a high level of expertise from the analyst. Water samples that are analyzed for labile species are not acidified (to avoid altering metal speciation) and must be analyzed promptly (Deaver and Rodgers, 1996). Although portable instrumentation was made in the 1980s, (Hall and Vaive, 1992a, 1992b), the inherent interferences may preclude ASV from gaining further advances in the field of metal analysis. This method is not commercially available and cost estimates are lacking. As a result of these characteristics, ASV is not yet and may never be suitable for routine biomonitoring of metals in water.

Ion Selective Electrodes (ISEs)

ISEs are electrochemical half-cells in which potential differences caused by the activity of a specific ion can be determined by measurement. They consist of metal electrodes invested with different materials (i.e., semiconductors, glass, ion exchangers) and are immersed in a solution containing the ions to be determined. These ions distribute themselves between electrode and solution phase. The potential difference is selectively dependant on the activity of the ions to be determined (Fresenius et al., 1988). Ion Selective Electrodes (ISE) have been used to differentiate between free and bound forms of metals (Gardiner, 1974; Sunda and Hanson, 1979). ISEs directly measure free ion concentrations, however, they generally lack sensitivity for use in natural waters (Hart, 1981; Lund, 1986).

There are only a few solid state ISEs that can be used to measure free metal ion activities for trace metals: Ag, Pb, Cu, Cd, Zn, and Hg (Fresenius et al.,1988). The Ag electrode is generally not very useful, as this element is extremely insoluble in natural water, whereas the Cu and Cd electrodes are most useful (Nordstrom, 1996). Gardiner (1974) found that results from the Cd specific electrode were very reproducible provided the system was allowed to reach equilibrium, because rapid pH changes were found to cause a temporary departure from the equilibrium value.

Unfortunately, ISEs suffer from a number of problems which hinder their general usefulness: interference from other major ions (i.e., sodium [Na], magnesium [Mg] and chloride [Cl]) present in natural waters (Lund, 1986); temperature variations; and electrode surface reactions (Suave et al., 1995). For example, the Cu and Pb specific electrode are susceptible to interference in brackish or marine waters (Sunda and Hanson, 1987; Lund, 1986).

Despite these shortcomings, ISE technology is rapidly evolving. For example, polymer membrane-based ion selective electrodes have been developed for the determination of metal ions, such as Pb and Cd. These electrodes represent a generic approach to chemical sensing, as their response characteristics rely on ion-extraction and complexation equilibria in the organic membrane phase. By chemically tailoring selective complexing agents within the membrane, the specificity and lifetime of these electrodes can be optimized for a given analytical task (Bakker et al.,1994)

Further advances in this field include the development of ion-selective bulk optodes (e.g. Pb). ISEs and optodes are based on similar components. Optodes, however, make use of optical sensors with fibre optic technology. The detection limit of the lead optode ($10^{-13.1}$M) was found to be much lower than that of the electrode ($10^{-9.2}$ M), but with sample activities of less than 10-11.5 M, response times of more than 60 have been observed with EDTA as a metal ion buffer. This is possibly due to a slow decomplexation step of the EDTA-Pb^{2+} complex (Bakker et al.,1993).

This is by no means a complete review of the advances in ISEs. This field is currently evolving and better ISE technologies may be available in the next 10 years (Ian Sharpe, pers. comm.). Generally, because of problems with interferences and lack of sufficient sensitivity, ISEs do not yet readily lend themselves to reliable speciation measurements in natural waters (Florence et al.,1983; Lund, 1986). Although a search on the Internet will reveal that some companies are working with ISEs, they are not used commercially (on a large scale) for metals analysis, and cost estimates are lacking.

Ion Exchange Resins and Ion Chromatography (IC)

Ion Chromatography (IC) is based on the theory that free metal forms present in water have a higher affinity for an ion exchange resin than complexed metal species; therefore, free metal ions will bind to the column while the complexed forms pass through. The separated metals are then quantified using traditional analytical methods (i.e., ICP-MS). One type of resin used in measuring the free metal ion is the Chelex -100 resin (Liu and Ingle, 1989; Pesavento and Biesuz, 1995). Typically, a filtered (0.45 μm) water sample is stirred with Chelex-100 resin in a beaker or is passed through a column containing the resin. The resin retains hydrated metal ions and weakly complexed or bound metal species that disassociate (Liu and Ingle, 1989).

Unfortunately, due to the retention of bound metal species, Chelex-100 is believed to overestimate the free metal concentration (Florence et al., 1983; Pesavento and Biesuz, 1995). Various other resins have been attempted but all overestimate the free ion concentration. IC technologies are still evolving and better IC methods may be

forthcoming in the next three years. Current price estimates are approximately $250 per metal analyzed.

Bioassay Data

Using laboratory toxicity tests, some research has attempted to evaluate the concentration of free metal ion that causes a toxic response to aquatic organisms at sublethal levels (i.e. below the detection limit of present physical/chemical methods) (e.g., Borgmann, 1981). In this study, the free Cu^{2+} ion concentrations that affected growth of freshwater copepod populations were estimated by comparing metal toxicity before and after addition of a complexing agent of known complexing capacity. In theory, the metal ion concentration can be determined from the displacement of the toxicity curve after addition of a weak complexing agent with a known stability constant. These toxicity tests rely on the assumption that the toxicity of the free metal ion does not change as the complexation and toxicity of total metal changes. Therefore, the general applicability of this approach to predict the amount of free metal ion causing a toxic response needs to be verified. These type of toxicity tests would complement the fish gill models (See section 2.4.3) currently being developed by others.

1.7 Summary of Analytical Technologies

Three requirements must be met in order for the previously mentioned alternative technologies to be useful tools in monitoring metals in the receiving environment:

- cost effectiveness
- availability and ease of use
- scientific validity.

Most of the alternative technologies are not commercially available and price estimates are lacking. Our review of current alternative technologies reveals the following limitations:

1. Anodic stripping voltammetry is direct in nature, highly sensitive for some metals (e.g., Cu, Zn, Cd, and Pb), but it is time consuming, requires a high level of operator expertise, and has problems with measurement error.

2. Only a few solid state Ion Selective Electrodes (ISEs) exist, with Cd and Cu selective electrodes being the most useful. The limited number of ISEs in existence, the lack of commercial availability and their limited detection sensitivity limits their use for water quality monitoring. However, development of this technology is changing rapidly and

it is possible that within the next 10 years ISE techniques may be used in routine aquatic effects monitoring.

3. Ion Chromatography is essentially a separation technique and requires further quantitative analysis (i.e., ICP-MS), which increases cost.

4. Chelex resins are available, but are believed to overestimate the free metal fraction.

5. Information on the bioassay approach is limited and the technique is not scientifically proven or widely used.

Currently, the majority of accredited analytical laboratories are using more established methods of measuring total/dissolved metals in freshwater, such as ICP/AAS techniques (CAEAL, 1995). This should continue until some of these alternatives are further developed or new ones are proven.

4.0
DETECTION LIMITS AND BIOLOGICAL EFFECTS

Analytical technology is rapidly improving our ability to accurately detect trace metals in water to extremely low concentrations. For example, a frequently cited goal in Canada is to analyze trace metals in receiving waters to detection limits equal to or lower than 1/10 of the corresponding CCME (or provincial) water quality guideline for the protection of aquatic life. For many parameters this requires the use of technology capable of achieving detection limits in the parts-per-trillion range. Therefore, the question arises: Is our ability to detect (or predict) significant biological effects in the receiving environment compromised if receiving water samples are not analyzed to ultra-low detection limits? The objective of this section is to address the need for ultra-low detection limits in routine monitoring to detect changes in water quality related to measurable biological effects in the receiving environment.

1.8 Definitions and Terminology

The detection limit associated with a test method is determined based on the measurement uncertainty (standard deviation) at zero (low) analyte concentration. The minimum detection limit is the concentration of an element that gives a signal equal to twice the peak-to-peak noise level of the baseline. In other words, the minimum detection limit is the lowest concentration that can be detected. The measurement of peak-to-peak noise level and signal level is shown in Figure 4-1. The signal is measured from its base at midpoint of the noise component along the slightly slanted baseline. There are wide variations in the detection limits for different chemical elements. This variation is attributable to differences in atomization efficiencies and the differing absorptivities of various elements (Harris, 1987). Detection limits may also vary depending on instrument sensitivity, levels of blank contamination, and/or matrix interferences.

Different detections limits have been defined based on relative measures of variation and reliability (i.e., lower uncertainty) of the reported test result.The following levels of detection limits have been used based on definitions provided by McQuaker (1997). The Method Detection Limit (MDL) is defined as the lowest concentration at which individual measurement results for a specific analyte are statistically different from a blank with a specified confidence level for a given method and representative matrix (Figure 4-2). As such, it is the measured response at which there is a stated probability (i.e., 95 or 99%) that the analyte is present in a sample. Consequently, there remains a level of probability (i.e., 5 or 1%) that analyte concentrations near the MDL will not be detected. The Reliable

Detection Limit (RDL) is the lowest analyte concentration required to be present in the sample to ensure detection and is equal to twice the MDL.

Figure 1-1. Measurement of the peak-to-peak noise level and signal level as indicated by an AAS sample graph.

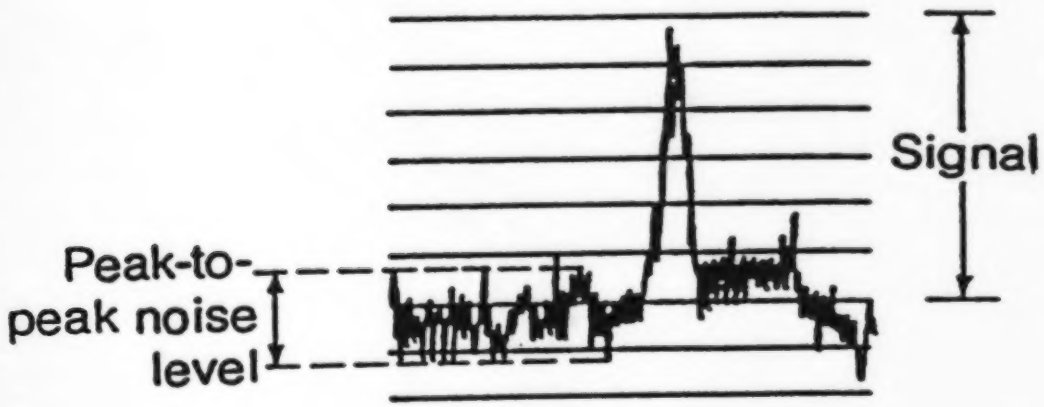
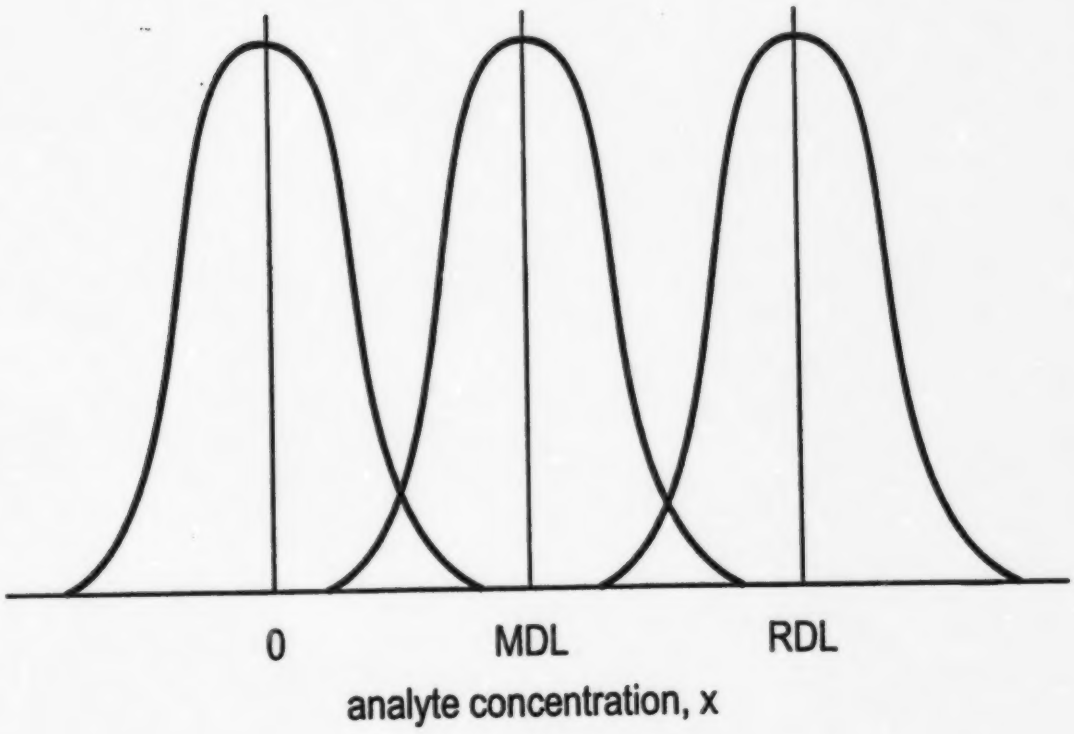


Figure 1-2. Schematic illustration of Method Detection Limit (MDL) and Reliable Detection Limit (RDL) (McQuaker, 1997).



Since MDLs and RDLs depend on laboratory precision, sample matrix, and methodology, they can vary quite a bit even for the same element. For example, MDLs for zinc using the same analytical technique may be different among laboratories due to differences in precision. Published MDLs must be considered only as approximate values since they are based on ideal samples (water blanks) which lack matrix and other effects often encountered in environmental samples. As such, it is recommended that detection limits include multiple defining modifiers so as to precisely define both the type of detection limit and the type of sample matrix used to determine the detection limit (Clark and Whitfield, 1994).

1.9 How Do Detection Limits Relate To Toxic Effects?

A mine will monitor its effluent for metals of concern. Typically, a mine in Canada will conduct baseline studies before initiating operation and conduct monitoring studies during operation. This approach provides information on whether the mine has a negative impact on the receiving environment. The assessment of biological effects from mining is often based on comparing monitoring water quality results to established criteria deemed acceptable for the protection of aquatic life. In determining whether the 1/10 level of detection limit to criterion ratio should be required to assess biological effects, the following 3 questions are discussed:

1. Is it feasible?

2. What is needed to ensure analytical confidence that reported non-detect results do not exceed acceptable levels.

3. What is the ecological risk (i.e., what is the need to ensure that generic criteria are not exceeded)?

1.9.1 Is It Feasible?

For many metals, satisfying the convention of analyzing analytes in receiving waters to detection limits equal to or lower than 1/10 of the corresponding CCME or BCMELP water quality guidelines does not necessitate the use of ultra-low detection limits (MDLs and RDLs). Table 4-1 provides a comparison of CCME and BCMELP criteria values for metal parameters with method and reliable detection limits for various analytical methods. For metals where criteria values depend on hardness and pH, values were calculated for "worst-case" conditions to illustrate the lower range of criteria. The method detection limits identified as achievable are the median reporting detection limits provided by

laboratories participating in the CAEAL (Canadian Association for Environmental Laboratories)

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Table 4-1. Comparison of Canadian fresh water aquatic life criteria values with achievable analytical detection limits (MDL and RDL).

	Criteria(ug/L)		Method Detection Limits [1] (ug/L)					MDL:Criteria [4]	RDL:Criteria
ELEMENT	CCME[2]	BCMELP[3]	FAAS	HGAAS	GFAAS	CVAAS	ICP-MS		
Aluminum	5	5	50	-	5	-	1	1:5	1:2.5
Antimony	-	20	-	2	1	-	0.1	1:200	1:100
Arsenic	5	5	-	2	1	-	1	1:5	1:2.5
Cadmium	0.01	0.01	2	-	0.2	-	0.05	1:0.2	1:0.1
Chromium	2	2	20	-	1	-	0.05	1:40	1:20
Copper	2	2	10	-	1	-	0.02	1:100	1:50
Iron	300	300	20	-	5	-	6	1:50	1:25
Lead	1	3	30	-	1	-	0.05	1:20	1:10
Manganese	-	100	10	-	1	-	0.05	1:2,000	1:1,000
Mercury	0.1	0.02	-	-	-	0.05	-	1:0.4	1:0.2
Molybdenum	-	1000	-	-	1	-	0.1	1:10,000	1:5000
Nickel	25	25	20	-	1	-	0.2	1:125	1:63
Selenium	1	1	-	2	1	-	1	1:1	1:0.5
Silver	0.1	0.05	-	-	0.1	-	0.05	1:1	1:0.5
Zinc	30	7	10	-	0.5	-	0.2	1:35	1:18

1 Method detection limits are the median reporting detection limits of laboratories participating in the CAEAL (Canadian Association for Environmental Laboratories) accreditation program. The detection limits (with the exception of mercury) are for dissolved metals (McQuaker, 1997).

2 CCME values from (CCREM 1987), CCME (1996a), and CCME (1996b). Criteria values represent the most sensitive freshwater conditions.

3 BCMELP values from BCMELP (1995), BCMELP (1 996) and Nagpal (1997). Criteria values represent the most sensitive freshwater conditions.

4 Ratios have been derived based on the most sensitive aquatic life criteria value and method detection limits associated with ICP-MS (i.e., shaded values)

Table 4-1. Comparison of Canadian fresh water aquatic life criteria values with achievable analytical detection limits (MDL and RDL).

	Criteria(ug/L)		Method Detection Limits [1] (ug/L)					MDL:Criteria [4]	RDL:Criteria
ELEMENT	CCME[2]	BCMELP[3]	FAAS	HGAAS	GFAAS	CVAAS	ICP-MS		
Aluminum	5	5	50	-	5	-	1	1:5	1:2.5
Antimony	-	20	-	2	1	-	0.1	1:200	1:100
Arsenic	5	5	-	2	1	-	1	1:5	1:2.5
Cadmium	0.01	0.01	2	-	0.2	-	0.05	1:0.2	1:0.1
Chromium	2	2	20	-	1	-	0.05	1:40	1:20
Copper	2	2	10	-	1	-	0.02	1:100	1:50
Iron	300	300	20	-	5	-	6	1:50	1:25
Lead	1	3	30	-	1	-	0.05	1:20	1:10
Manganese	-	100	10	-	1	-	0.05	1:2,000	1:1,000
Mercury	0.1	0.02	-	-	-	0.05	-	1:0.4	1:0.2
Molybdenum	-	1000	-	-	1	-	0.1	1:10,000	1:5000
Nickel	25	25	20	-	1	-	0.2	1:125	1:63
Selenium	1	1	-	2	1	-	1	1:1	1:0.5
Silver	0.1	0.05	-	-	0.1	-	0.05	1:1	1:0.5
Zinc	30	7	10	-	0.5	-	0.2	1:35	1:18

1 Method detection limits are the median reporting detection limits of laboratories participating in the CAEAL (Canadian Association for Environmental Laboratories) accreditation program. The detection limits (with the exception of mercury) are for dissolved metals (McQuaker, 1997).

2 CCME values from (CCREM 1987), CCME (1996a), and CCME (1996b). Criteria values represent the most sensitive freshwater conditions.

3 BCMELP values from BCMELP (1995), BCMELP (1 996) and Nagpal (1997). Criteria values represent the most sensitive freshwater conditions.

4 Ratios have been derived based on the most sensitive aquatic life criteria value and method detection limits associated with ICP-MS (i.e., shaded values)

accreditation (McQuaker, 1997). Lower method detection limits may be achievable by some individual laboratories.

In general, ICP-MS is needed to detect levels in the environment lower than criteria values; however, this is considered to be a relatively feasible option given its high ease of use and reasonable cost (Table 3-1). Detection limits associated with ICP-MS generally satisfy the 1/10 ratio convention for many metals (Table 4-1). However, ratios of MDL:criterion values may be less than 1/10 and in some cases, even exceed criteria for 6 metals (i.e., Al, As, Cd, Hg, Se and Ag) when the most sensitive criterion is applied.

1.9.2 What is Needed to Ensure Analytical Confidence?

Analytical variation increases as analyte concentrations become smaller. As such, measuring concentrations in proximity to detection limits will significantly increase the uncertainty in results. When levels are too low, the signal to noise ratio is minimal and there is higher uncertainty in results. The key requirement for determining biological effects is the use of adequate analytical detection limits which can, with a high statistical probability, detect analytes at concentrations of concern. In other words, there is a very low probability that concentrations measured at or below detection limits would exceed acceptable levels.

It can be argued from a statistical perspective that ratios of 1/2 RDL (i.e., 1/4 MDL) should afford an adequate level of analytical assurance assuming that all factors of variance (e.g., matrix interferences) have been incorporated and the level of variance follows a linear relationship. As such, requirements for appropriate levels of detection limits should be based on meeting the necessary analytical confidence in results rather than an arbitrary number (1/10) set in relation to established criteria values. Consideration should also be given to placing increased responsibility on analytical laboratories to demonstrate adequate levels of precision.

1.9.3 What is the Ecological Risk?

Risk of Exposure

Discharges from different sites vary in the amount as well as the type of metals present (i.e., not all metals are present in every discharge). If there is a very low risk that effluents would contain those specific analytes with associated low criteria, it may be argued that it is not necessary to continually analyze to ultra-low detection limits during routine monitoring studies. By taking into account the probability that specific analytes are even present, this approach tailors monitoring efforts in accordance with the specific characteristics of the effluent.

Risk of Effects

Referenced Canadian and provincial criteria have been derived to safeguard ambient water quality and are not representative of specific water bodies. In order to safeguard against Aworst-case conditions@, ambient criteria are often driven by those conditions that result in the highest toxicity to aquatic organisms. However, these Aworst-case@ conditions are not applicable to all receiving water bodies. In addition, aquatic environments in geologic areas that are naturally mineralized may have elevated metal levels in water (Chapman et al., 1996), and organisms in these ecosystems may be acclimated or adapted to high metal concentrations. Consequently, we recommend applying site-specific criteria for aquatic environments receiving mine effluent. In this regard, detection limit requirements should be determined based on site-specific criteria. Initially, this may require a mine to conduct more extensive water quality surveys, coupled with chemical speciation modeling and toxicity tests, to determine any modifications to existing ambient criteria that may be appropriate for the receiving environment. However, this approach should enable a mine to develop a more cost-effective and focussed monitoring program over the long-term, and would afford a level of protection appropriate to the site-specific conditions.

In summary, we recommend that detection limits for routine monitoring be established on a site-specific basis. Specifically, decisions regarding detection limit requirements should consider the associated ecological risk based on the likelihood of exposure and the severity of associated effects given the site-specific conditions. For analytes of concern, detection limits should be based on the analytical requirements which ensure an acceptable confidence level that reported non-detects are significantly different than effect concentrations (i.e., generic criteria if applicable or site-specific criteria). As such, increased responsibility should be placed on analytical laboratories to demonstrate acceptable levels of analytical assurance (i.e., Atruly@ defined levels of detection limits) for low analyte concentrations. Finally, detection limits need to be precisely defined and adequate assurance given with test results that all factors of variance have been incorporated.

5.0
SUMMARY AND RECOMMENDATIONS

- The current definition of dissolved metals is inadequate. We recommend that a workshop be held that addresses this definition, discusses the relationship between dissolved and total metals and toxicity, and standardizes the approach to filtering water for metal analyzes in Canada that minimizes contamination.

- We suggest that alternative approaches to evaluating the nature of the chemical environment in waters receiving mining effluent be evaluated. For example, chemical equilibrium modeling could be used as a tool to predict metal species and their probability of exerting a negative biological effect in the receiving environment.

- A risk based approach should be used to establish detection limits on a site-specific basis. For analytes of concern, detection limits should be based on analytical requirements needed to ensure that reported non-detects are significantly different than effect concentrations (i.e., generic criteria if applicable or site-specific criteria).

- At the moment, there are no alternative technologies for characterizing metals (e.g., anodic stripping voltammetry) that are of practical use in monitoring metals in the receiving environment. Although various alternative approaches can measure the free metal ion, they all have a number of problems that limit their practical application. We recommend that grant money be directed to further develop these approaches (i.e., especially ISEs) so that they can be of general use in monitoring metals in the receiving environment.

6.0
REFERENCES

Allison, J. D., D. S. Brown, and K. J. Novo-Gradac. 1991. MINTEQA2/PRODEFA2, a geochemical assessment model for environmental systems. Version 3.0 User's Manual. U. S. Environmental Protection Agency, Washington, D. C.

Anderson, D.M., and F.M.M. Morel. 1978. Copper sensitivity of *Gonyaulax tamarensis*. Limnol. Oceanogr. 23: 283-295.

Anderson, M.A., and F.M.M. Morel. 1982. The influence of aqueous iron chemistry on the uptake of iron by the coastal diatom *Thalassiosira weissflogii*. Limnol. Oceanogr. 27: 789-843.

Bakker, E., M.Willer, and E. Pretsch. 1993. Detection limit of ion-selective bulk optodes and corresponding electrodes. Anal. Chim. Acta 282: 265-271.

Bakker, E., R.K. Meruva, E. Pretsch, and M.E. Meyerhoff. 1994. Selectivity of polymer-based ion-selective electrodes: self consistent model describing the potentiometric response in mixed ion solutions of different charge. Anal. Chem. 66: 3021-3030.

BCMELP (B.C. Ministry of Environment, Lands and Parks). 1994. British Columbia Environmental Laboratory Manual for the Analysis of Water, Wastewater, Sediment, and Biological Materials. Laboratory Services, Environmental Protection Department, Victoria, B.C.

BCMELP. 1995. Approved and working criteria for water quality - 1995. Water Quality Branch, Environmental Protection Department. Victoria, B.C.

BCMELP. 1996. Ambient water quality criteria for silver. Water Quality Branch, Environmental Protection Department, Victoria, BC.

Borgmann, U. 1981. Determination of free metal ion concentrations using bioassays. Can. J. Fish. Aquat. Sci. 38: 999-1002.

Buchwalter, D.B., G. Linder, and L.R. Curtis. 1996. Modulation of cupric ion activity by pH and fulvic acid as determinants of toxicity in *Xenopus laevis* embryos and larvae. Environ. Toxicol. Chem. 15:568-573.

CAEAL. 1995. Directory of Certified Laboratories. Canadian Association for Environmental Analytical Laboratories (CAEAL) Inc.

Campbell, P.G.C. 1995. Interactions between trace metals and aquatic organisms: A critique of the free-ion activity model. In: Metal Speciation and Bioavailability in Aquatic Systems. A. Tessier, and D.R. Turner (eds). John Wiley & sons Ltd.

Campbell, P.G.C., and P.M. Stokes. 1985. Acidification and toxicity of metals to aquatic biota. Can. J. Fish. Aquat. Sci. 42: 2034-2049.

CCME (Canadian Council of Ministers of the Environment). 1996a. Canadian Water Quality Guidelines updates (May 1996): Cadmium. In: CCREM 1987, Appendix XXI.

CCME.1996b. Canadian Water Quality Guidelines updates: Arsenic. In CCREM 1987, Appendix XXIII. *In Press.*

CCREM (Canadian Council of Resource and Environment Ministers). 1987. Canadian Water Quality Guidelines. Prepared by the Task Force on Water Quality Guidelines of the Canadian Council of Resource and Environment Ministers.

Chapman, P.M., C.A. McPherson, and C. MacCay. 1996. Natural pollution and NPDES permit process: the Red Dog Mine, Alaska. Poster presented at the 17th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Washington, D.C.

Clark, M.J.R., and P.H. Whitfield. 1994. Conflicting perspective about detection limits and about the censoring of environmental data. Water Resour. Bulletin. 30: 1063-1079.

Dallinger, R., F. Prosi, H. Segner, and H. Back. 1987. Contaminated food and uptake of heavy metals by fish: a review and a proposal for further research. Oecologia (Berlin) 73:91-98.

Davies, P.H. 1986. Toxicology and chemistry of metals in urban runoff. pp 60-78. In: Urban Runoff Quality Impact and Quality Enhancement Technology. B. Urgonas, and L.A. Roesner (eds). Proceedings of an Engineering Foundation Conference, Henniker, New Hampshire. American Society of Civil Engineers, New York.

Davies, P.H., and S. Brinkman. 1993. Statewide fish research: water pollution studies. Study No F-33. Colorado Division of Wildlife. Research Center. Fort Collins, Colorado. 39 pp + appendices.

Deaver, E., and J.H. Rodgers, Jr. 1996. Measuring bioavailable copper using anodic stripping voltametry. Environ. Toxicol. Chem. 15:1925-1930.

Demayo, A. 1992. Average concentrations of elements in the earth=s crust as cited by Dr. M.J.R. Clark, B.C. Ministry of Environment, Lands and Parks, Victoria, B.C. In: Lide, D.R. (ed), CRC Handbook of chemistry and Physics, 73rd Edition. p. 14-7.

Eisler, R. 1988. Lead hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Fish Wildl. Serv. Biol. Rep. 85 (1.14). 134 pp.

Erickson, R.J., D.A. Benoit, V.R. Mattson, H.P. Nelson Jr., and E.N. Leonard. 1996. The effects of water chemistry on the toxicity of copper to fathead minnows. Environ. Toxicol. Chem. 15:181-193.

Florence, T.M., B.G. Lunsden, and J.J. Fardy. 1983. Evaluation of some physico-chemical techniques for the determination of the fraction of dissolved copper toxic to the marine diatom *Nitzschia closterium*. Anal. Chim. Acta. 151: 281-295.

Fresenius, W., K.E. Quentin, and W. Schneider (eds.). 1988. Water analysis: a practical guide to physico-chemical, chemical and microbiological water examination and quality assurance. Springer-Verlag, Berlin Heidelberg, Germany.

Gardiner, J. 1974. The chemistry of cadmium in natural water: a study of cadmium complex formation using the cadmium specific-ion electrode. Water Res. 8: 23-30.

Giesy Jr, J.P., G.J. Leversee, and D.R. Williams. 1977. Effects of naturally occurring aquatic organic fractions on cadmium toxicity to *Simocephalus serrulatus* (Daphnidae) and *Gambusia affinis* (Poeciliidae). Water Res. 11:1013-1020.

Hall, G.E.M. 1992. Inductively coupled plasma mass spectrometry in geoanalysis. J. Geochem. Explor. 44: 201-249.

Hall, G.E.M. and J.E. Vaive. 1992a. Determination of gold in geological samples by anodic stripping voltammetry at field locations. Chem. Geol. 102: 41-52.

Hall, G.E.M. and J.E. Vaive. 1992b. Application of a field portable anodic stripping voltammeter to the analysis of sulphide selective leaches and waters. Chem. Geol. 97:295-306.

Hare, L., and A. Tessier. 1996. Predicting animal cadmium concentrations in lakes. Nature 380: 430-432.

Harris, D.C. 1987. Quantitative Chemical Analysis. 2nd. edition. Freeman & Co., New York, New York.

Hart, B.T. 1981. Trace metal complexing capacity of natural waters: a review. Environ. Technol. Lett. 2: 95-110.

Horowitz, A.J., K.R. Lum, J.R. Garbarino, G.E.M. Hall, C. Lemieux, and C.R. Demas.1996. Problems associated with using filtration to define dissolved trace element concentrations in natural water samples. Environ. Sci. Technol. 30: 954-963.

Jackim, E., J. M. Hamlin, and S. Sonis. 1970. Effect of metal poisoning on five liver enzymes in the killyfish (*Fundulus heteroclistus*). J. Fish. Res. Board Can. 27: 383-390.

Jacobs, R.M. 1996. Techniques employed for the assessment of metals in biological systems. pp 69-79. In: Toxicology of Metals. L.W. Chang, L.Magos, and T.Suzuki (eds). CRC Lewis Publishers, Boca Raton, New York, New York.

Janes, N., and R.C. Playle. 1995. Modeling silver binding to gills of rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 14:1847-1858.

Jones, B.F., V.C. Kennedy, and G.W. Zellweger. 1974. Comparison of observed and calculated concentrations of dissolved Al and Fe in stream water. Water Resour. Res. 10:791-793.

Kiffney, P. M., and W. H. Clements. 1993. Bioaccumulation of heavy metals by benthic invertebrates at the Arkansas River, Colorado. Environ. Toxicol. Chem. 12: 1507-1517.

Kimball, B.A., D.M. McKnight, G.A. Wetherbee, and R.A. Harnish. 1992. Mechanisms of iron photoreduction in a metal-rich acidic stream (St. Kevin Gulch, Colorado, U.S.A.). Chem. Geol. 96: 227-239.

Kimball, B.A., E. Callender, and E.V. Axtmann. 1995. Effects of colloids on metal transport in a river receiving acid mine drainage, upper Arkansas River, Colorado, U.S.A. Appl. Geochem. 10: 285-306.

Kramer, J.R., W. Davidson, K. L. Godtfredsen, J.S. Meyer, M. Perdue, E. Tipping, D. Van de Meent, and J. Westall. 1996. Chemical speciation and metal toxicity in surface freshwaters. pp 5.1-5.17. In: Reassessment of Metals Criteria for Aquatic Life Protection. H. Bergman, E. Dorward-King, H. Allen, D. DiToro, R. Erickson, J. Matice, M. Reiley. (eds). SETAC, Pensacola, Florida.

Liu, Y., and J. D. Ingle Jr. 1989. Automated two-column ion exchange system for determination of the speciation of trace metals in natural waters. Anal. Chem. 61: 525-529.

Lund, W. 1986. Electrochemical methods and their limitations for the determination of metal species in natural waters. pp 533-561. In: The Importance of Chemical "Speciation" in Environmental Processes. M. Bernhard, F.E. Brinckman, and P.J. Sadler (eds). Dahlem Konferenzen 1986, Springer-Verlag, Berlin, Heidelberg, Germany.

Luoma, S.N. 1983. Bioavailability of trace metals to aquatic organisms - a review. Sci. Total Environ. 28:1-22.

Luoma, S.N., and E.A. Jenne. 1976. Factors affecting the availability of sediment-bound cadmium to the estuarine, deposit-feeding clam, *Macoma balthica*. Pp 283-290. In: Radioecology and Energy Resources. C.E. Cushing (ed). Ecol. Soc. Am. Spec. Publ. No. 1, Dowden, Hutchinson and Ross, Stroudsbourg, Pennsylvania.

Luoma, S.N., and E.A. Jenne. 1977. The availability of sediment-bound cobalt, silver and zinc to a deposit-feeding clam. Pp 213-230. In: Biological Implications of Metals in the Environment. H. Drucker, and R.E. Wildung (eds). ERDA Symp. Ser. No. 42, Energy Research and Development Administration, Washington, D.C.

Mach, M.H., B. Nott, J.W. Scott, R.F. Maddalone, and N.T. Whiddon. 1996. Metal speciation: survey of environmental methods of analysis. Water. Air. Soil. Pollut. 90: 269-279.

McQuaker, N.R., 1997. Technical evaluation on water quality design and analysis (Revision 2.0). Prepared for the Aquatic Effects Technology Evaluation Program.

Meador, J.P. 1991. The interaction of pH, dissolved organic carbon, and total copper in the determination of ionic copper and toxicity. Aquat. Toxicol. 19:13-32.

Nagpal, N. 1997. Personal communication (telephone conversation with M. Daykin, EVS Consultants Ltd., Vancouver, BC). Water Quality Branch, Environmental Protection Department, Ministry of Environment, Lands and Parks, Victoria, BC. 8 May.

Ney, J. J., and J. H. Van Hassel. 1983. Sources of variability in accumulation of heavy metals by fishes in a roadside stream. Arch. Environ. Contam. Toxicol. 12: 701-706.

Nordstrom, K.D. 1996. Trace metal speciation in natural waters: computational vs analytical. Water. Air. Soil. Pollut. 90: 257-267.

Pagenkopf, G.K., C.R. Rosemarie, and R.V. Thurston. 1974. Effect of complexation on toxicity of copper to fishes. J. Fish. Res. Board Can. 31: 462-465.

Pesavento, M., and R. Biesuz. 1995. Simultaneous determination of total and free metal ion concentration in solution by sorption on iminodiacetate resin. Anal. Chem. 67: 3558-3563.

Prosi, F. 1981. Heavy metals in aquatic organisms. In: Metal Pollution in the Aquatic Environment. U. Forstner and G. T. W. Whitman (eds.), Springer-Verlag, Berlin, Heidelberg, Germany. pp. 271-323.

Robin, J. 1988. Analysis of water by emission spectrometry with an inductively-coupled plasma source. In: The Determination of Trace Metals in Natural Waters. T.S. West, and H.W. Nurnberg (eds). Blackwell Scientific Publications, London.

Rubeska, I. 1988. Determination of trace elements in natural waters by atomic absorption spectrometry. pp 91-103.In: The Determination of Trace Metals in Natural Waters. T.S. West, and H.W. Nurnberg (eds). Blackwell Scientific Publications, London. England.

Schecher, W. D., and D. C. McAvoy. 1992. MINEQL+: A software environment for chemical equilibrium modeling. Computers, Environment, and Urban Systems 16: 65-76.

Sharpe, I. 1997. Personal communication (telephone conversation with Alena Fikart, EVS Consultants Ltd., Vancouver, BC). BC Minstry of Environment, Skeena Region.

Suave, S., M.B. McBride, and W.H. Hendershot. 1995. Ion-selective electrode measurements of copper (II) activity in contaminated soils. Arch. Environ. Contam. Toxicol. 29:373-379.

Sunda, W.G., and R.R.L. Guillard. 1976 The relationship between cupric ion activity and the toxicity of copper to phytoplankton. J. Mar. Res. 34: 511-529.

Sunda, W.G., and P.K. Hanson. 1979. Chemical speciation of copper in river water: Effect of total copper, pH, carbonate, and dissolved organic matter. pp 148-179. In: Chemical Modeling in Aqueous Systems. E.A. Jenne (ed). ACS Symposium Series 94. American Chemical Society, Washington, D.C.

Sunda, W.G., and P.K. Hanson. 1987. Measurement of free cupric ion concentration in seawater by a ligand competition technique involving copper sorption onto C_{18} SEP-PAK cartridges. Limnol. Oceanogr. 32: 537-551.

Taylor, H.E., and A.M. Shiller. 1995. Mississippi River methods comparison study: implications for water quality monitoring of dissolved trace elements. Environ. Sci. Technol. 29:1313-1317.

U.S. EPA (U.S. Environmental Protection Agency). 1995. Method 1669. Sampling ambient water for trace metals at EPA water quality criteria levels. U.S. Environmental Protection Agency, Washington, DC. 36 pp.

U.S. EPA. 1996. The Metals Translator: Guidance for Calculating a Total Recoverable Permit Limit from a Dissolved Criterion. U.S. Environmental Protection Agency, Washington, DC. 60 pp.

Welsh, P.G., J.F. Skidmore, D.J. Spry, D.G. Dixon, P.V. Hodson, N.J. Hutchinson, and B.E. Hickie. 1993. Effect of pH and dissolved organic carbon on the toxicity of copper to larval fathead minnow (*Pimephales promelas*) in natural lake waters of low alkalinity. Can. J. Fish. Aquat. Sci. 50: 1356-1362.

Winner, R.W. 1984. The toxicity and bioaccumulation of cadmium and copper as affected by humic acid. Aquat.Toxicol. 5:267-274.

Winner, R.W. 1985. Bioaccumulation and toxicity of copper as affected by interactions between humic acid and water hardness. Water Res. 19: 449-455.

Witters, H.E., S.V. Puymbroeck, A.J.H.X. Stouthart, and S.E. Wendelaar Bonga. 1996. Physicochemical changes of aluminum in mixing zones: mortality and physiological disturbances in brown trout (*Salmo trutta L.*). Environ. Toxicol. Chem.15: 986-996.

Woodward, D.F., W.G. Brumbaugh, A.J. DeLonay, E.E. Little, and C.E. Smith.1994. Effects on rainbow trout fry of a metals-contaminated diet of benthic invertebrates from the Clark Fork River, Montana. Trans. Am. Fish. Soc. 123:51-66.